

GARY L. FAHNENSTIEL, TYRONE R. PATTON, HUNTER J. CARRICK, and
MICHAEL J. MCCORMICK

Great Lakes Environmental Research Laboratory
2205 Commonwealth Blvd. Ann Arbor, MI 48105, USA

Diel Division Cycle and Growth Rates of *Synechococcus* in Lakes Huron and Michigan¹

key words: *Synechococcus*, diel division pattern, frequency of dividing cells (FDC) method, duration of division

Abstract

A clone of *Synechococcus* isolated from Lake Huron and natural populations of *Synechococcus* from lakes Huron and Michigan were studied in 1989 to examine the diel division cycle and to provide estimates of the *in situ* growth rate based on the frequency of dividing cells (FDC) method. Cultured populations of *Synechococcus* exhibited a consistent diel division pattern with a midday/afternoon (1100–1800 h) peak in the percent of dividing cells. The maximum percent of dividing cells varied among cultures (8–27 %) and was related to the growth rate. A small fraction of dividing cells (3–5 %) remained throughout the dark period, suggesting that some cells were arrested in the doublet stage prior to division. The duration of division (t_d) ranged from 2.6–4.9 h, with a 3.7 h mean for cultures with growth rates $\geq 0.34 \text{ d}^{-1}$ but increased to 8 h at a lower growth rate of 0.20 d^{-1} .

The diel division pattern for natural populations was very similar to the laboratory clone; an afternoon peak (1400–2100 h) in dividing cells and a small fraction of dividing cells (2–5 %) remained during the dark period. The maximum percent of dividing cells for natural populations ranged from 6–10 %. *In situ* growth rates, determined from the FDC and assuming a constant t_d of 3.7 h, ranged from $0.30\text{--}0.42 \text{ d}^{-1}$. The FDC method may provide accurate estimates of *in situ* growth, particularly in environments where the growth rate is $> 0.34 \text{ d}^{-1}$, but in lakes Huron and Michigan where growth rates can be lower and t_d values may increase, FDC-growth rates must be viewed with caution.

1. Introduction

Chroococcoid cyanobacteria of the genus *Synechococcus* are important components of pelagic ecosystems in both freshwater and marine environments (see review of STOCKNER and ANTIA 1986). The importance of *Synechococcus* increases in the more oligotrophic environments where it can contribute as much as 95 % to the total primary production (ITURRIAGA and MARRA 1988). In the Laurentian Great Lakes, *Synechococcus* can contribute as much as 20 % of the primary production during the period of thermal stratification (FAHNENSTIEL *et al.* 1986; FAHNENSTIEL *et al.* 1991). Because of its importance in many environments, there is much interest in its autecology, particularly, with reference to production, growth and loss rates. Yet, most rate measurements (production, growth, loss, etc.) of *Synechococcus* and other phytoplankton are made in bottles that may produce significant containment effects and thus, inaccurate estimates (VENRICK *et al.* 1977;

FAHNENSTIEL and CARRICK 1988). Techniques that do not require incubations in bottles are preferred.

One promising approach for estimating *in situ* growth rates of individual phytoplankton populations is the mitotic index based on the fraction of cells undergoing mitosis (MCDUFF and CHISHOLM 1982). For prokaryotic cells such as *Synechococcus*, *in situ* growth rates can be inferred simply from the frequency of dividing cells. This approach has been tested for several marine populations of *Synechococcus* and appears to be a useful technique for estimating *in situ* growth rates (CAMPBELL and CARPENTER 1986).

In this study we examined the diel division cycle of an isolate of *Synechococcus* from Lake Huron to determine the feasibility of using the frequency of dividing cells (FDC) to estimate *in situ* growth rates. We also examined the diel division cycle of field populations of *Synechococcus* from lakes Huron and Michigan to provide estimates of *in situ* growth.

2. Materials and Methods

Laboratory Experiments. An isolate of *Synechococcus* from Lake Huron was used to explore the diel division cycle under controlled conditions and to examine the relationship between the FDC and growth rate. Several cultures of this isolate were grown in BG-11 culture medium (STANIER *et al.* 1971) with a 15:9 light:dark cycle and variable light (12, 16, 20, 27, 47, 75 $\mu\text{Ein} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and temperature (13, 14, 16, 20°C) levels. Each culture was grown under these specific conditions for several generations. A few days (1–3) prior to the start of a diel experiment, a fraction (10 ml) of the exponentially growing culture was diluted with 140 ml of BG-11 medium. When the culture resumed log phase growth, as determined by *in vivo* fluorescence, the diel experiment began and picoplankton samples were collected every 1–2 h for 24–48 h and preserved with glutaraldehyde (1% final conc.) buffered with sodium cacodylate (0.1 M final conc.).

Preserved samples were kept cold (ca. 5 °C), and replicate slides were immediately prepared and frozen (–20 °C); slides were counted within a week to minimize errors due to fading of auto-fluorescence. Total and dividing *Synechococcus* cells were enumerated on a Leitz Laborlux epi-fluorescent microscope (excitation = 450 nm). *Synechococcus* was defined as single (noncolonial) cells of approximately 1- μm in diameter, which fluoresced yellow when excited with blue light. For enumerating dividing cells, the division phase was defined as the time from which an invagination in the cell wall was evident until two distinct cells could be identified. A minimum of 600 cells was enumerated for each sample. Growth rates of these cultures were determined from changes in abundance during the experiment.

The duration of division (t_d) was calculated from laboratory experiments of the isolated clone, with the following equation:

$$t_d = \frac{1}{n\mu} \sum_{i=1}^{i=n} \ln(1 + f_i) \quad (1)$$

where n = number of sampling intervals, μ = growth rate (d^{-1}), and f_i = fraction of dividing cells for each sampling interval.

Field Experiments. Field sampling was conducted in northern Lake Huron and southern Lake Michigan during the period of thermal stratification (June–October 1989). A Lagrangian sampling program was used where water samples for diel experiments were collected in the vicinity of a satellite-tracked-drifter of known windage (McCORMICK *et al.* 1985). Once the drifter was deployed, picoplankton samples were collected every 1–2 h with 5-L Niskin bottles. These samples were preserved, prepared, and enumerated for both total and dividing *Synechococcus* cells, as described above. Equation 1 was used to estimate *in situ* growth rates by assuming a t_d value (from laboratory cultures) and solving for μ . The following assumptions are implied with the FDC technique: (1) all cells are active and (2) t_d is similar for all cells of the population and does not vary with environmental conditions, i.e. growth rate.

Temperature was measured with an electronic bathythermograph and bucket thermometer. Chlorophyll concentrations were determined fluorometrically on 90% acetone extracts (STRICKLAND and PARSONS 1972).

Small inocula growth (SIG) experiments were used to provide an *in vitro* estimate of growth. A 4-L polycarbonate bottle with prefiltered lakewater ($<0.2 \mu\text{m}$, Gelman Mini-Capsule) was inoculated with filtered lakewater ($<3 \mu\text{m}$ filtrate, Nuclepore filter) and incubated at ambient light and temperature in a decktop incubator for 24 h. Growth rates were calculated from the measured changes in abundance within the sample bottle.

3. Results and Discussion

A strong diel pattern in the division cycle was noted for all cultures. The percent of dividing cells was low during the dark period, increased during the light period, and reached a maximum in the afternoon approximately 5–12 h after initial light exposure (Fig. 1; Table 1). The maximum number of dividing cells for each experiment ranged

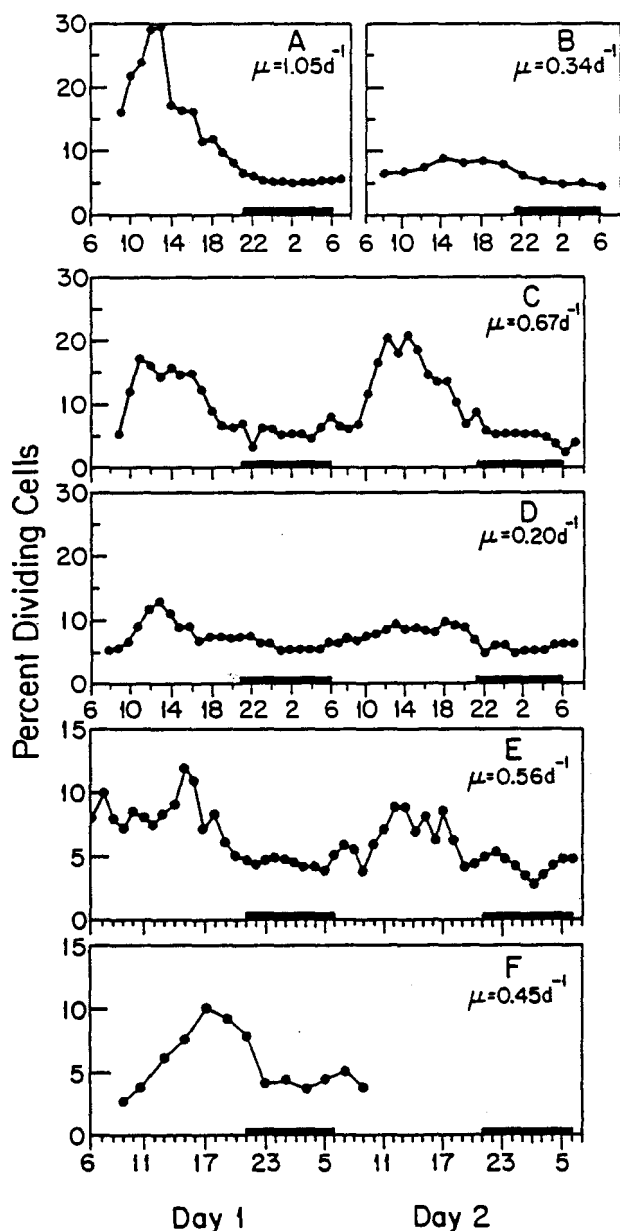


Figure 1. Diel pattern of percent dividing cells for cultures of a clone of *Synechococcus* isolated from Lake Huron and grown under different light (L , $\mu\text{Einst} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and temperature (T , $^{\circ}\text{C}$) regimes. (A) growth rate = 1.05 d^{-1} , $T = 14$, $L = 75$, (B) growth rate = 0.34 d^{-1} , $T = 16$, $L = 16$, (C) growth rate = 0.67 d^{-1} , $T = 14$, $L = 47$, (D) growth rate = 0.20 d^{-1} , $T = 20$, $L = 12$, (E) growth rate = 0.56 d^{-1} , $T = 20$, $L = 27$, (F) growth rate = 0.45 d^{-1} , $T = 14$, $L = 20$. Horizontal bar indicates dark period.

Table 1. Characteristics of the diel division cycle for laboratory cultures of *Synechococcus* isolated from Lake Huron, grown at various temperatures and light levels. The maximum percent of dividing cells (FDC Max.), the time of day of this maximum (Time), and the duration of division (t_d) are indicated for each culture.

Light Level ($\mu\text{Einst} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Temp. ($^{\circ}\text{C}$)	Growth rate (d^{-1})	FDC Max. (%)	Time (h)	t_d (h)
12	20	0.20	12.9/9.9	1300	8.3
16	16	0.34	8.7	1400	4.2
20	14	0.45	10.2	1700	4.9
27	20	0.56	12.1/9.1	1600/1400	2.6
47	13	0.67	17.0/20.9	1100/1400	3.4
75	14	1.05	29.1	1200	3.1

from 8–21 % and was related to the growth rate (Fig. 2, $y = 3.3X + 0.03$, $R^2 = 0.73$, $p = 0.003$). In future studies, this relationship may be used to estimate *in situ* growth rates since it is far easier to sample in the mid-afternoon for the FDC maximum than the entire diel cycle. However, the scatter in this relationship at low growth rates will limit its usefulness. It should be emphasized that this relationship was not used to estimate growth rates in this study, rather *in situ* growth rates were calculated from the total number of dividing cells over a 24 h period (equation 1).

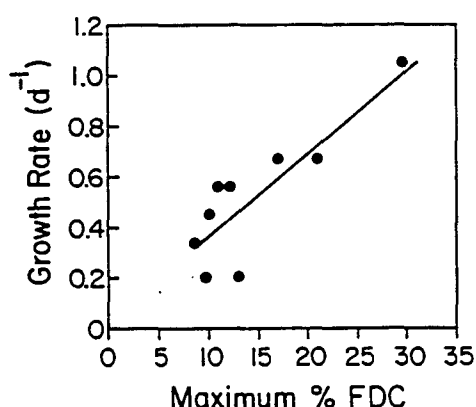


Figure 2. Growth rate and maximum percent of dividing cells for cultures of a *Synechococcus* clone isolated from Lake Huron.

The diel pattern of dividing cells of our clone is very similar to the patterns observed for most other *Synechococcus* clones that have been studied under laboratory conditions (CAMPBELL and CARPENTER 1986; WATERBURY *et al.* 1986; ARMBRUST *et al.* 1989). In previous studies, the maximum division peak generally occurred in the middle-to-late part of the light period, with a strong relationship existing between the maximum percent of dividing cells and growth rate. A relatively constant fraction of cells was found to remain in the division cycle during the night, suggesting that t_d was not similar for all cells in the populations, as some cells were arrested in the doublet stage prior to division (ARMBRUST *et al.* 1989).

The duration of division was relatively similar (2.6–4.9 h) at growth rates of 0.34–1.05 d^{-1} , but increased to 8.3 h at the lowest growth rate (0.20 d^{-1} , Table 1). The mean t_d for cultures with growth rates of $\geq 0.34 \text{ d}^{-1}$ was 3.7 h. The relationship we found between t_d and growth rate is remarkably similar to that observed for *Synechococcus* clone WH 7803, where t_d values were approximately 3 h for cultures with growth rates $> 0.4 \text{ d}^{-1}$ but increased to as high as 12 h at lower growth rates (CAMPBELL and CARPENTER 1986). Because t_d values varied with growth rate, the FDC technique will be most useful when growth rates are $\geq 0.34 \text{ d}^{-1}$.

Field populations of *Synechococcus* exhibited a similar diel division pattern to the cultured clone. The percent of dividing cells was low during the night and increased during the day, reaching maximum values during the afternoon/early evening period (1400–2100 h) (Table 2; Fig. 3). The maximum percent of dividing cells ranged from

Table 2. Diel division cycle characteristics and *in situ* growth rates of natural populations of *Synechococcus* from lakes Huron and Michigan during the period of thermal stratification. Depth, temperature, maximum percent of dividing cells (FDC Max.), time of maximum, and *in situ* growth rate (eq. 1, t_d of 3.7 h) are indicated for each sample.

Lake	Date	Depth (m)	Temp. (°C)	FDC Max. (%)	Time of Max. (h)	Growth Rate (d ⁻¹)
Mich.	12/6/89	2	9.2	9.3	1800	0.42
		5	9.2	6.7	1400	0.38
		10	9.0	6.5	2000	0.34
		20	6.0	6.0	1400	0.30
Mich.	11/7/89	3	19.2	10.3	1700	0.36
		8	19.1	7.0	1700	0.32
		20	6.2	6.8	2100	0.32
Huron	30/7/89	2	20.2	8.5	1600	0.37
		5	20.1	7.4	1800	0.32
		15	12.8	6.2	1800	0.33
Mich.	13/9/89	5	18.5	6.9	1800	0.37
Mich.	5/10/89	5	13.2	8.1	1600/1800	0.42

6.0–10.3 %, which is relatively similar to values for laboratory cultures with growth rates of 0.2–0.45 d⁻¹ (Table 1; Fig. 1). A low percentage of dividing cells, 2–5 %, was found during the night.

The division cycle observed for the field populations from lakes Huron and Michigan was similar to that for *Synechococcus* populations from coastal marine environments, where afternoon/early evening maxima were found (WATERBURY *et al.* 1986; CARPENTER and CAMPBELL 1988), but different from the night time maxima or no-phased division cycle observed for populations from the North Atlantic Ocean (CAMPBELL and CARPENTER 1986; PRÉZELIN *et al.* 1987). *Synechococcus* populations from the Sargasso Sea appear to exhibit a variable diel division pattern (WATERBURY *et al.* 1986; PRÉZELIN *et al.* 1987).

The maximum number of dividing cells for populations from lakes Huron and Michigan is on the low end of values reported for marine populations and is probably due to the lower growth rates of *Synechococcus* from lakes Huron and Michigan (FAHNENSTIEL *et al.* 1991). Our range of maximum percent dividing cells (6–10 %) is lower than the values of 10–24 %, 10–30 %, 8–32 %, and 5–15 % reported for marine populations by CAMPBELL and CARPENTER (1986), WATERBURY *et al.* (1986), CARPENTER and CAMPBELL (1988) and PRÉZELIN *et al.* (1987), respectively.

In situ growth rates estimated from the FDC (equation 1), assuming a t_d of 3.7 h, ranged from 0.30–0.42 d⁻¹ (Table 2). Because of the uncertainty of t_d values at growth rates < 0.34 d⁻¹ (Table 1), these *in situ* rates must be viewed with some caution. The assumption of constant t_d values for all environmental conditions (i.e. growth rates) is probably the most troublesome assumption for the widespread use of the FDC technique. For all clones of *Synechococcus* studied to date, the assumption of a constant t_d cannot be justified at low growth rates (Table 1; CAMPBELL and CARPENTER 1986).

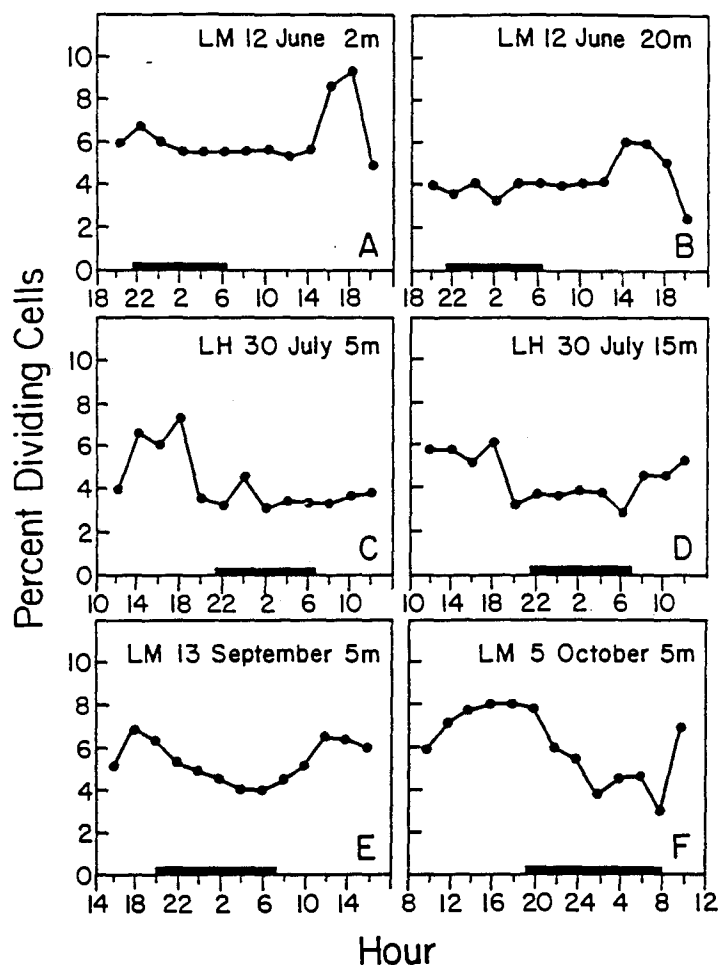


Figure 3. Examples of the diel pattern of percent dividing cells for field populations of *Synechococcus* from lakes Huron (LH) and Michigan (LM) during the period of thermal stratification in 1989. Horizontal bar indicates dark period.

The other assumptions of the FDC technique (i.e. constant t_d for all cells within population and all cells are active) are probably of lesser concern. A constant fraction of cells were arrested during the dark period (Fig. 3) suggesting that t_d was not similar for all cells within the population. However, because the percent of cells arrested in the dark was similar for both field and laboratory populations (2–5%), equation (1) should still produce consistent estimates. Although we did not evaluate the possibility of dormancy within our populations we do not believe that dormancy is a common feature of phytoplankton communities within the euphotic zone. Using track autoradiography to estimate species-specific production, we have consistently observed that most phytoplankton populations were uniformly labeled (G. FAHNENSTIEL, unpubl. data).

Despite the uncertainty regarding low growth rates from the FDC method, our *in situ* estimates were relatively similar to *in vitro* growth rates from SIG experiments (Table 3; paired- $t = 0.2$, $p = 0.86$, $n = 4$). The good agreement among estimates should not be taken as unequivocal support for the accuracy of FDC estimates. First, this comparison was limited ($n = 4$). Second and probably more important, SIG estimates are likely to be poor measures of *in situ* phytoplankton growth in Lake Michigan. During the period of thermal stratification, significant containment effects have been found for incubations lasting

Table 3. Comparison of *in situ* FDC and *in vitro* SIG growth rates from Lake Michigan (FDC growth rates were calculated from eq. 1, $t_d = 3.7$ h).

Date	Depth (m)	FDC (d^{-1})	SIG (d^{-1})
12/6/89	5	0.38	0.34
11/7/89	3	0.36	0.30
13/9/89	5	0.37	0.57
5/10/89	5	0.42	0.26

24 h (FAHNENSTIEL and SCAVIA 1987; FAHNENSTIEL and CARRICK 1988). Thus, the accuracy of FDC-based growth rates from lakes Huron and Michigan is still open to question, particularly at the lowest growth rates.

4. Summary and Conclusions

An isolate of *Synechococcus* from Lake Huron, grown under a variety of light and temperature conditions, exhibited a pronounced diel division cycle with an afternoon peak in dividing cells. The maximum percent of dividing cells ranged from 8–21 % and was related to the growth rate. The duration of division (t_d) was similar (2.6–4.9 h) at growth rates $\geq 0.34 d^{-1}$ but increased to 8 h at a lower growth rate ($0.2 d^{-1}$). Similar diel division patterns (afternoon/early evening maxima in dividing cells) were found for field populations of *Synechococcus* in lakes Huron and Michigan during the period of thermal stratification. *In situ* growth rates calculated by the frequency of dividing cells (FDC), assuming a t_d value of 3.7 h, ranged from 0.30–0.42 d^{-1} . Due to the relatively low growth rates of *Synechococcus* in lakes Huron and Michigan and the uncertainty of t_d values at these low growth rates, the FDC method has potential limitations in lakes Huron and Michigan.

5. Acknowledgements

We would like to thank J. STOCKNER and an anonymous reviewer for their comments on the manuscript. Contribution No. 743 Great Lakes Environmental Research Lab.

6. References

- ARMBRUST, E. V., J. D. BOWEN, R. J. OLSON, and S. W. CHISHOLM, 1989: Effect of light on the cell cycle of a marine *Synechococcus* strain.—Appl. Env. Microbiol. 55: 425–432.
- CAMPBELL, L., and E. J. CARPENTER, 1986: Diel patterns of cell division in marine *Synechococcus* spp. (Cyanobacteria): Use of the frequency of dividing cells technique to measure growth rate.—Mar. Ecol. Prog. Ser. 32: 139–148.
- CARNEY, H. J., and G. L. FAHNENSTIEL, 1987: Quantification of track and grain density autoradiography and evaluation of ^{14}C loss on preservation.—J. Plankton Res. 9: 41–50.
- CARPENTER, E. J., and L. CAMPBELL, 1988: Diel patterns of cell division and growth rates of *Synechococcus* spp. in Long Island Sound.—Mar. Ecol. Prog. Ser. 47: 179–183.
- FAHNENSTIEL, G. L., and H. J. CARRICK, 1988: Primary production in lakes Huron and Michigan: *in vitro* and *in situ* comparisons.—J. Plankton Res. 10: 1273–1283.
- FAHNENSTIEL, G. L., H. J. CARRICK, and R. ITURRIAGA, 1991: Physiological characteristics and food web dynamics of *Synechococcus* in lakes Huron and Michigan.—Limnol. Oceanogr. 36: 219–234.
- FAHNENSTIEL, G. L., and D. SCAVIA, 1987: Dynamics of Lake Michigan phytoplankton: Primary production and growth.—Can. J. Fish. Aquat. Sci. 44: 499–508.

- FAHNENSTIEL, G. L., L. SICKO-GOAD, D. SCAVIA, and E. F. STOERMER, 1986: Importance of picoplankton in Lake Superior.—*Can. J. Fish. Aquat. Sci.* 43: 235–240.
- ITURRIAGA, R., and J. MARRA, 1988: Temporal and spatial variability of chroococcoid cyanobacteria *Synechococcus* spp. specific growth rates and their contribution to primary production in the Sargasso Sea.—*Mar. Ecol. Prog. Ser.* 44: 175–181.
- MCCORMICK, M. J., A. H. CLITES, and J. E. CAMPBELL, 1985: Water tracking ability of satellite-tracked drifters in light winds.—*Mar. Technol. Soc. J.* 19: 12–17.
- MCDUFF, R. E., and S. W. CHISHOLM, 1982: The calculation of *in situ* growth rates of phytoplankton populations from fractions of cells undergoing mitosis: A clarification.—*Limnol. Oceanogr.* 27: 783–788.
- PRÉZELIN, B. B., H. E. GLOVER, and L. CAMPBELL, 1987: Effects of light intensity and nutrient availability on diel patterns of cell metabolism and growth in populations of *Synechococcus* spp.—*Mar. Biol.* 95: 469–480.
- STANIER, R. Y., R. KUNISAWA, R. MANDEL, G. COHEN-BAZIRE, 1971: Purification and properties of unicellular blue-green algae (Order Chroococcales).—*Bacteriol. Rev.* 35: 171–205.
- STOCKNER, J. G., and N. J. ANTIA, 1986: Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective.—*Can. J. Fish. Aquat. Sci.* 43: 2472–2503.
- STRICKLAND, J. D. H., and T. R. PARSONS, 1972: A Practical Handbook of Seawater Analysis, 2nd Ed.—*Bull. Fish. Res. Bd. Can.* No. 167.
- VENRICK, E. L., J. R. BEERS, and J. F. HEINBOKEL, 1977: Possible consequences of containing microplankton for physiological rate measurements.—*J. Exp. Mar. Biol. Ecol.* 26: 55–76.
- WATERBURY, J. B., S. W. WATSON, F. W. VALOIS, and D. G. FRANKS, 1986: Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*.—*Can. Bull. Fish. Aquat. Sci.* 214: 71–120.

